CANCER GENOMICS

NTRK Fusions Identified in Pediatric Tumors: The Frequency, Fusion Partners, and Clinical Outcome

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PURPOSE Neurotrophic tyrosine receptor kinase (NTRK) fusions have been described as oncogenic drivers in a variety of tumors. However, little is known about the overall frequency of NTRK fusion in unselected pediatric tumors. Here, we assessed the frequency, fusion partners, and clinical course in pediatric patients with NTRK fusion–positive tumors.

PATIENTS AND METHODS We studied 1,347 consecutive pediatric tumors from 1,217 patients who underwent tumor genomic profiling using custom-designed DNA and RNA next-generation sequencing panels. NTRK fusions identified were orthogonally confirmed.

RESULTS AND DISCUSSION NTRK fusions were identified in 29 tumors from 27 patients with a positive yield of 2.22% for all patients and 3.08% for solid tumors. Although *NTRK2* fusions were found exclusively in CNS tumors and *NTRK1* fusions were highly enriched in papillary thyroid carcinomas, *NTRK3* fusions were identified in all tumor categories. The most canonical fusion was *ETV6-NTRK3* observed in 10 patients with diverse types of tumors. Several novel NTRK fusions were observed in rare tumor types, including *KCTD16-NTRK1* in ganglioglioma and *IRF2BP2-NTRK3* in papillary thyroid carcinomas. The detection of an NTRK fusion confirmed the morphologic diagnosis including five cases where the final tumor diagnosis was largely based on the discovery of an NTRK fusion. In one patient, the diagnosis was changed because of the identification of an *ETV6-NTRK3* fusion. One patient with infantile fibrosarcoma was treated with larotrectinib and achieved complete pathologic remission.

CONCLUSION NTRK fusions are more frequently seen in pediatric tumors than in adult tumors and involve a broader panel of fusion partners and a wider range of tumors than previously recognized. These results highlight the importance of screening for NTRK fusions as part of the tumor genomic profiling for patients with pediatric cancer.

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INTRODUCTION

Rearrangements involving the neurotrophic tyrosine receptor kinase (NTRK) genes *NTRK1*, *NTRK2*, and *NTRK3* encode fusion proteins containing the intact NTRK kinase domain that are oncogenic drivers of a histologically diverse group of tumors. NTRK rearrangements involving dozens of genes have been described and lead to constitutively unregulated activation of TRK kinases and downstream pathways.[1] In children, the incidence of NTRK fusions is high (> 90%) in certain tumors such as infantile fibrosarcoma (IF), congenital mesoblastic nephroma, and secretory carcinoma, and lower (5%-26%) in pediatric papillary thyroid carcinomas (PTCs)[2] and in a subset of pediatric gliomas.[3–5] By contrast, NTRK fusions are rarely identified in GI stromal tumors, melanoma, lung adenocarcinoma, acute leukemia, and soft-tissue sarcomas with a range of histologic morphologies.[5–8] The dramatic and durable objective responses in cancers harboring NTRK fusions have led the (US) Food and Drug Administration to approve first-generation oral NTRK inhibitors, larotrectinib and entrectinib, for use in patients of various ages with advanced solid tumors regardless of tumor histology.[9–11] Other NTRK inhibitors are in clinical development including selitrectinib (LOXO-195), taletrectinib (DS-6051b),[12] and repotrectinib (TPX-005).[13,14] Clinical trials are ongoing to determine the optimal use of these drugs in children (Children's Oncology Group Trial ADVL1823, ClinicalTrials.gov identifier: NCT03834961). We conducted a single institution retrospective review of tumors with NTRK fusions identified by next-generation sequencing at the Children's Hospital of Philadelphia (CHOP) to assess the frequency, fusion partners, and clinical course in infants, children, and adolescents with NTRK fusion–positive tumors to highlight the importance of

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTENT

Key Objective

NTRK fusions are tumor-agnostic or age-independent biomarkers that identify patients suitable for treatment with the US Food and Drug Administration–approved TRK inhibitors. The spectrum of NTRK fusions has been described in adult cancers, but is incompletely known for pediatric cancers. This single institutional study examined the frequency, fusion partner, and clinical outcome of NTRK fusions in a large cohort of unselected patients with pediatric cancer.

Knowledge Generated

Our study demonstrated that NTRK fusions are more frequent in pediatric tumors and involve a broader panel of fusion partners and a wider range of tumors those than previously recognized and highlights pediatric cancers in which NTRK fusions are more or less likely to occur.

Relevance

This study provided important findings regarding NTRK fusion–positive pediatric cancers and cancers where NTRK fusions are rarely seen. The data can help prioritize pediatric tumors for NTRK fusion testing to enable implementation of targeted treatment in children and adolescents with cancer.

NTRK fusion screening in patients with pediatric cancer for personalized tumor stratification and treatment.

PATIENTS AND METHODS

We evaluated 1,347 consecutive tumors from 1,217 pediatric patients including 604 male and 613 female patients from CHOP for NTRK fusions as part of routine comprehensive clinical sequencing from March 2016 to September 2019. For patients whose tumor harbored an NTRK fusion, chart review was conducted to determine treatment received and clinical outcome. Length of follow-up time was recorded. Histology results were reviewed and tabulated. Immunohistochemistry was performed in a subset of cases using pan-TRK antibody (Abcam, Cambridge, UK, 1:100 dilution) (Table 1).

Fusion gene detection was performed using the CHOP Cancer Fusion Panel as previously described.[15] Briefly, target-specific primers covering 673 exons were custom designed to identify known fusion genes and potential novel fusion genes associated with 110 cancer genes using anchored multiplex polymerase chain reaction (PCR) technology (ArcherDX, Inc, Boulder, CO). Total RNA (or total nucleic acid from formalin-fixed paraffin-embedded (FFPE) samples) was extracted from the tumor samples and reverse-transcribed into cDNA. Libraries were constructed using Archer Universal RNA Reagent Kit v2 for Illumina. Barcoded libraries were pooled and sequenced on Illumina HiSeq platform using 150 bp paired-end sequencing (Illumina, San Diego, CA). Sequence data were analyzed and visualized using Archer Analysis software using the JBrowse genome browser (Evolutionary Software Foundation, Berkeley, CA). All fusions, when identified in our laboratory for the first time, were confirmed by nested PCR followed by Sanger sequencing.

Mutations (single nucleotide variants [SNVs] and small insertion/deletions [indels]) and copy number alterations (CNAs) were evaluated by using the CHOP Hematological Malignancy Panel (CHMP) or Solid Tumor Panel (CSTP) as described previously.[16] Briefly, genomic DNA was extracted from the tumor samples. Libraries were prepared using probes targeting 118 genes (CHMP) or 238 genes (CSTP), respectively, and sequenced on Illumina HiSeq platform using 150 bp paired-end sequencing. Sequence data were analyzed using the home brew software ConcordS v2 (for SNVs and indels) and NextGENe v2 Next-Generation Sequencing Analysis Software (for CNAs; SoftGenetics, LLC, State College, PA). Clinically significant variants including SNVs, indels, and CNAs were confirmed by Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), real-time PCR, or droplet digital PCR (ddPCR) when necessary. All genomic coordinates are based on Genome Reference Consortium Human Build 37 (GRCh37).[17]

RESULTS AND DISCUSSION

The Spectrum of NTRK Fusions in Pediatric Cancers

We performed fusion gene studies of 1,347 consecutive tumors from 1,217 pediatric patients to evaluate the spectrum of NTRK fusions in the largest cohort of pediatric tumors to date. NTRK fusions were identified in 29 tumors from 27 patients with a positive yield of 2.22% for all tumors and 3.08% for solid tumors (Tables 1 and 2, Fig. 1). Of the 27 NTRK fusions, five were novel (not previously published) at the time of discovery (Table 1). The age of NTRK-positive patients ranged from 0.1 to 17 years with 17 females and 10 males. NTRK fusions were detected in 13% of PTCs (10 of 76), 1.9% of CNS tumors (7 of 364), 1.8% of non-CNS, non-PTC solid tumors (8 of 435), and 0.4% of hematologic malignancies (2 of 472) (Table 2). Specifically, *NTRK2* fusions were found exclusively in CNS tumors, and *NTRK1* fusions were highly enriched in PTCs, whereas *NTRK3* fusions were identified in all tumor categories (Table 2 and Fig. 1). Some fusions were recurrently identified in histologically distinct tumor types, such as *ETV6-NTRK3* in six PTCs, two IFs, one secretory carcinoma of salivary glands, and one congenital glioblastoma; as well as

TABLE 1. Summary of NTRK Fusions Identified in the Cohort With Treatment and Follow-Up

Patient ID	Histologic Diagnosis	Age at Pathologic Diagnosis (y)	Sex	Fusion Gene	Impact of Identified NTRK Fusions on Diagnosis[b]	Stage/Anatomic Location At Diagnosis	Surgical Resection	Radiotherapy/ Chemotherapy	Outcome
CNS									
1	Pilocytic astrocytoma, anaplastic	2	F	*KANK1-NTRK2*[a]	1	Local, cerebellum	Gross total; repeat gross total 2 y later	No	Recurrence 2 years postresection; no repeat recurrence at 31 mo
2	Astrocytoma, diffuse	12	F	*C2orf44-NTRK2*[a]	1	Local, tectum	No, biopsy only	No	No recurrence at 23 mo
3	Astrocytoma, diffuse	16	F	*QKI-NTRK2*	1	Local, temporal lobe	Gross total	No	No recurrence at 21 mo
4	Ganglioglioma	7	M	*KCTD16-NTRK2*[a]	1	Local, parietal lobe	Gross total	No	No recurrence at 11 mo
5	Ganglioglioma	9	M	*TRIM24-NTRK2*	1	Local, parietal/occipital lobes	Gross total	No	No recurrence at 10 mo
6	Mixed neuronal glial tumor NOS	11	M	*SPECC1L-NTRK2*[a]	1	Local, frontal lobe	Gross total	No	No recurrence at 34 mo
7	Congenital glioblastoma	0.3	F	*ETV6-NTRK3*	1	Local, posterior cerebral hemispheric	No	No	Death (multisystem failure)
Hematologic malignancy									
8	T-lymphoblastic lymphoma	5	F	*RBPMS-NTRK3*	1	Mediastinal mass, CNS 1, BM MRD negative	No	AALL1231-LIKE (arm A)	No recurrence at 21 mo
9	Myeloid Sarcoma	16	F	*TPM3-NTRK1*	1	Liver, CNS unknown, BM MRD–positive	N/A	ADE with GTMZ induction	Death during induction
Non-CNS non-PTC solid tumors									
10	Secretory carcinoma	3	M	*ETV6-NTRK3*	2	Local, right parotid, lymph node–negative	Gross total right-modified radical neck dissection	No; received focal proton radiation therapy	No recurrence at 44 mo
11	Myofibroblastic sarcoma, intermediate grade[c]	13	F	*TFG-NTRK3*	1	Local, thigh	Gross total	No	No recurrence at 46 mo
12	Malignant spindle cell tumor[c]	10	F	*RBPMS-NTRK3*	3	Local, Liver (porta hepatis)	Gross total (liver transplant)	2 cycles ifos/doxo, 2 cycles VDC	No recurrence at 39 mo
13	IF	0.2	M	*ETV6-NTRK3*	3	Local, tongue	Near total (positive margins)	No	No recurrence at 38 mo
14	IF	0.1	M	*ETV6-NTRK3*	3	Local, orbit	Near total (positive margins)	EE4A-LIKE (19 wk)	No recurrence at 27 mo
15	IF	0.5	M	*SPECC1L-NTRK3*	3	Local, triceps	Gross total	Larotrectinib	No recurrence at 6 mo
16	Epithelioid melanocytic tumor of uncertain malignant potential (cutaneous melanocytoma)	12	F	*PRDX1-NTRK1*	1	Local, ankle	Near total (positive margins)	No	No recurrence at 12 mo
17	Spindle cell tumor, low grade[c]	0.5	M	*STRN3-NTRK3*	3	Local, tongue	Near total (positive margins)	No	No recurrence at 9 mo
Thyroid tumors									
18	Papillary thyroid carcinoma, classic variant, multifocal	16	F	*ETV6-NTRK3*	1	T1bN0Mx	Negative margins	None	Remission: No; Progressive: No at 46 mo
19	Papillary thyroid carcinoma, diffuse sclerosing variant	9	F	*ETV6-NTRK3*	1	T1bN1bM0	Negative margins	RAI	Remission: yes; Progressive: No at 44 mo
20	Papillary thyroid carcinoma, diffuse sclerosing variant	9	M	*IRF2BP2-NTRK1*[a]	1	T4aN1bM1	Positive margins	RAI	Remission: yes; Progressive: yes at 43 mo
21	Papillary thyroid carcinoma, follicular variant, widely invasive	16	M	*ETV6-NTRK3*	1	T1bN1bM1	Positive margins	RAI	Remission: No; Progressive: No at 25 mo
22	Papillary thyroid carcinoma, with mixed follicular, papillary, and solid growth patterns	17	F	*ETV6-NTRK3*	1	T1bN1bMx	Negative margins	None	Remission: Yes; Progressive: no at 22 mo
23	Papillary thyroid carcinoma, with mixed follicular, papillary, and solid growth patterns	16	F	*ETV6-NTRK3*	1	T3aN1bM1	Positive margins	RAI	Remission: No; Progressive: No at 21 mo
24	Papillary thyroid carcinoma, with mixed papillary and micropapillary growth patterns	6	F	*SQSTM1-NTRK1*	1	T4aN1bM1	Positive Margins	RAI	Remission: No; Progressive: yes at 20 mo
25	Papillary thyroid carcinoma, tall cell variant	15	F	*TPR-NTRK1*	1	T4aN1bM1	Unknown, metastatic node resection	RAI	Remission: No; Progressive: No at 18 mo
26	Papillary thyroid carcinoma, tall cell variant	15	F	*TPR-NTRK1*	1	T2N1bM0	unknown, metastatic node resection	RAI	Remission: No; Progressive: No at 11 mo
27	Papillary thyroid carcinoma, classic variant	12	F	*ETV6-NTRK3*	1	T1bN0Mx	Positive margin (minute, focal)	None	Remission: Yes; Progressive: No at 8 mo

Abbreviations: ADE, chemotherapy regimen including cytarabine, daunorubicin, and etoposide; BM, bone marrow; CNS 1, level 1 CNS involvement; GTMZ, gemtuzumab; MRD, minimal residual disease; RAI, radioactive iodine; VDC, chemotherapy regimen including vincristine, doxorubicin, and cyclophosphamide.

[a]Novel fusions (not previously published) at the time of discovery.

[b]Impact of identified NTRK fusions on diagnosis: 1—the NTRK fusions identified confirmed the clinical and/or pathological diagnoses; 2—the diagnosis was changed because of the identification of the NTRK fusion; 3—tumor diagnosis was largely based on the discovery of the NTRK fusion.

[c]These tumors now meet criteria for the diagnosis of NTRK-rearranged spindle cell neoplasm according to the Fifth edition of the Soft Tissue and Bone Tumors WHO Classification (2020).

TABLE 2. Distribution of Neurotrophic Tyr receptor kinase Fusion–Positive Cases in the Cohort

Histologic Diagnosis	Patients With *NTRK1* Fusions	Patients With *NTRK2* Fusions	Patients With *NTRK3* Fusions	Total No. of Patients With NTRK-Positive Fusions	Total No. of Patients (Tumors) Tested	Positive Rate (%)
CNS	0	6	1	7	338 (364)	2.07
Hematologic malignancy	1	0	1	2	405 (472)	0.49
Non-CNS nonpapillary thyroid carcinoma solid tumors	1	0	7	8	401 (435)	2.00
Thyroid tumors	4	0	6	10	73 (76)	13.70

RBPMS-NTRK3 in a spindle cell sarcoma and a T-lymphoblastic lymphoma. By contrast, different NTRK fusions were present in tumors with the same histological diagnosis, such as *KCTD16-NTRK2* and *TRIM24-NTRK2* in two male patients of similar age with ganglioglioma, WHO grade I (Table 1). Several novel NTRK fusions were observed in rare tumor types, such as *KANK1-NTRK2* in malignant anaplastic pilocytic astrocytoma,[18] *C2orf44-NTRK2* in diffuse astrocytoma, *IRF2BP2-NTRK1* in PTC, *KCTD16-NTRK2* in ganglioglioma, and *SPECC1L-NTRK2* in glioneuronal tumor[19] (Fig. 1). These data demonstrate that NTRK fusions are far more frequently seen in pediatric tumors than in adult tumors and involve a broader panel of fusion partners and a wider range of pediatric tumors than previously recognized.[20,21] With the recent (US) Food and Drug Administration approval of larotrectinib and entrectinib for the treatment of adult and pediatric NTRK-positive, unresectable solid tumors, identification of these fusions directly affects patient care.[22]

NTRK Fusions in Pediatric Carcinomas

NTRK fusions were detected in 10 pediatric PTCs (13%) including 6 patients with *ETV6-NTRK3* fusions, one diffuse sclerosing variant (Figs. 2E-F), two mixed follicular, papillary, and solid growth patterns, one widely invasive follicular variant (Fig. 2D), and two classic variants; two with *TPR-NTRK1* fusions, both tall cell variants; one with *SQSTM1-NTRK1* fusion showing a mixed classic and micropapillary growth patterns; and one patient with a novel *IRF2BP2-NTRK1* fusion at the time of discovery, encompassing a diffuse sclerosing PTC (Table 1). Finally, there was a single case of secretory carcinoma of salivary glands with the canonical *ETV6-NTRK3* fusion identified (Table 1). In PTC, tumorigenesis is associated with constitutive activation of the MAPK and PI3K signaling pathways secondary to somatic point mutations in *BRAF*, *PTEN*, *DICER1*, and *RAS* as well as fusions involving RET, ALK, and NTRK.[3,23] Gene fusions have been reported in both sporadic and radiation-induced PTCs and are more common in pediatric (50%-60%) compared with adult tumors (approximately 15%).[2,23] RET and NTRK fusions are the most common, reported in 25%-30% and approximately 10% (range, 0%-26%) of pediatric PTCs, respectively.[2,23] *NTRK3* fusions are usually observed more commonly in PTCs than *NTRK1* fusions,[2,23] which is similar to what we observed in our patient cohort. All the PTCs in our cohort were sporadic, none associated with

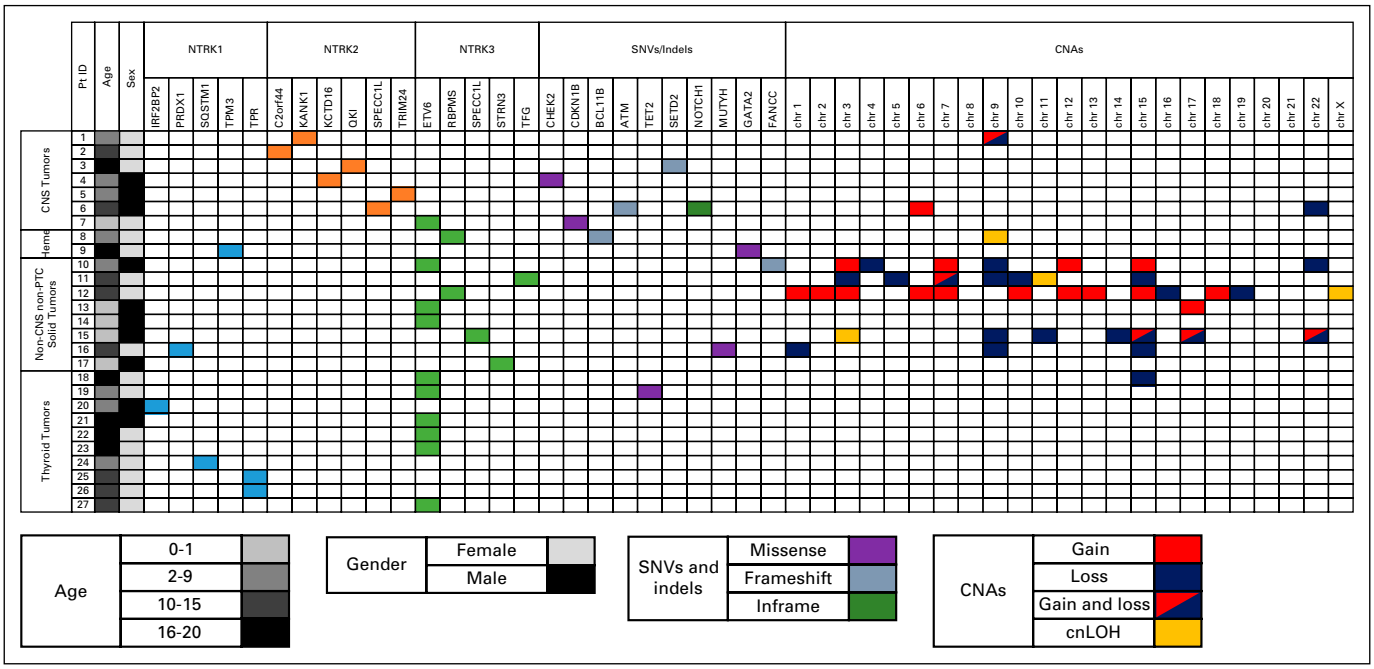

FIG 1. Summary of molecular findings of NTRK fusion–positive patients identified in the cohort at CHOP.

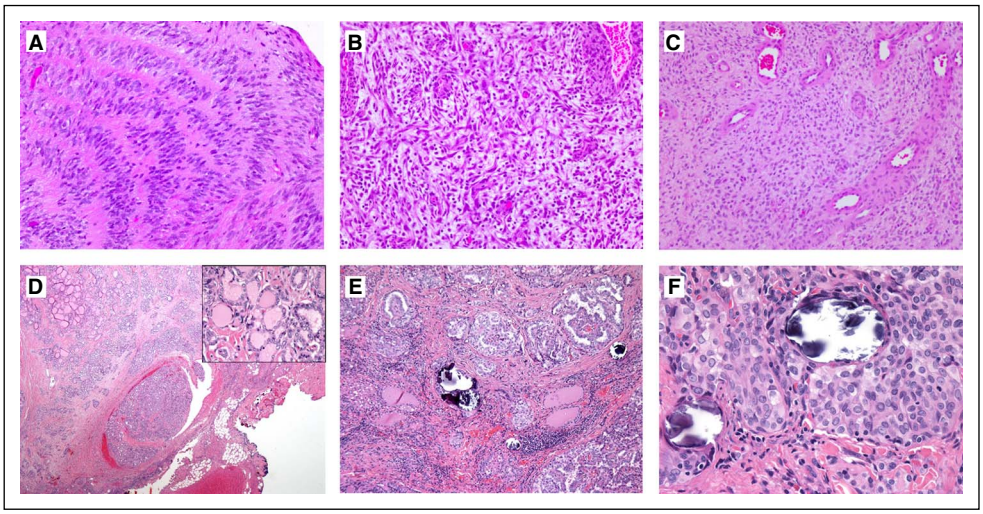

FIG 2. Representative examples of soft-tissue tumors (A-C) and papillary thyroid carcinoma (D-F). Case 12 with *RBPMS-NTRK3* tumor of the liver hilum showing nuclear palisading (A, H&E, 200×). Case 13 demonstrates an IF with canonical *ETV6-NTRK3* showing tissue culture–like growth with interspersed inflammatory cells (B, H&E 200×). Case 17 demonstrates a low-grade tumor with *STRN3-NTRK3* showing moderately cellular spindle to round cell proliferation with prominent vascular proliferation (C, H&E, 200×). Case 21 shows widely invasive follicular variant of papillary thyroid carcinoma with *ETV6-NTRK3* demonstrating extrathyroidal extension and angioinvasion (D, H&E, 50×) and nuclear features of papillary thyroid carcinoma (inset, 400×). Two cases demonstrate the diffuse sclerosing variant of papillary thyroid carcinoma showing solid and papillary groups infiltrating the thyroid parenchyma with numerous psammomatous calcifications (E, H&E, 100×). Case 19 on high power shows solid tumor cell groups (squamoid morphology) with psammomatous calcifications (F, H&E, 400×). H&E, hematoxylin and eosin staining.

previous exposure to radiation, and NTRK fusions were found in both prepubertal and pubertal patients across the spectrum of histological variance. In thyroid cancers, NTRK fusions were 100% specific for PTC and, of the 10 patients with PTC with NTRK fusions, the majority presented with invasive disease, characterized by lymphovascular permeation throughout the thyroid, extrathyroidal extension, lateral neck lymph node metastases (8 of 10 with N1b, 80%), extranodal extension, and pulmonary metastasis (5 of 10 with M1, 50%)[24] (Table 1). Within PTC tumors harboring an NTRK fusion and pulmonary metastasis, three were found to harbor an *NTRK1* fusion and two with an *NTRK3* fusion (Table 1 and Fig. 1). The data are limited but suggest that the presence of an NTRK fusion may have diagnostic, prognostic, and therapeutic significance in PTCs and may hold clinical utility for stratifying surgical and medical care. Standard therapy for advanced PTCs in children involves surgical resection of gross disease followed by radioactive-iodide (RAI) therapy. In a recent multicenter, open-label phase I or II study of larotrectinib for the treatment of pediatric patients with solid tumors, two children with advanced PTCs were treated for > 7 months and remained progression-free, although, unfortunately, the objective response to treatment was not reported.[25]

NTRK Fusions in Pediatric CNS Tumors

All the CNS tumors identified with NTRK fusions were either gliomas or mixed neuronal glial tumors. Most of the CNS tumors contained *NTRK2* fusions with different 5′ partners, except the single congenital glioblastoma with *ETV6-NTRK3* (Table 1 and Fig. 1). Four of the six *NTRK2* fusions were novel fusions at the time of discovery with different 5′-partner genes including *KANK1*, *C2orf4*, *KCTD16*, and *SPECC1L*. NTRK fusion genes have been described in pediatric low- and high-grade gliomas at a low prevalence,[26] although one study reported a finding of 40% of nonbrainstem high-grade gliomas in children younger than 3 years old containing an NTRK fusion gene (n = four of the 10 samples).[27] Six of the 7 NTRK fusion–positive CNS tumors were low-grade gliomas (LGG), which may be partially due to the higher frequency of LGG in our unselected pediatric cohort (approximately 45% of all CNS tumors). Our cohort demonstrates an increased prevalence of *NTRK2*-associated fusions within CNS tumors compared with those occurring extracranially (Table 1 and Fig. 1), which has been observed in prior studies.[28,29] The therapeutic implication of these fusions in pediatric brain tumor is at this time unclear based on limited available publications. Although the duration of follow-up in our study is limited, the majority of patients underwent standard of care for their CNS tumor subtype with resection of the primary tumor without recurrence. Specifically, five of the seven patients in our cohort have been managed surgically without adjuvant treatment, consistent with the natural history of these tumors without NTRK fusions. However, although the patient cohort has not required adjuvant therapy to date, given the potential for recurrence and the potential morbidity of repeat surgical resection, medical management targeting TRK may need to

TABLE 3. Morphologic and Immunohistochemical Features of Soft-Tissue NTRK-Fused Tumors

Patient ID	Initial Diagnosis	Age	Fusion	Distinctive Morphologic Features	Mitotic Rate (#/10 HPF)	Immunohistochemistry			
						SMA	CD34	S100	pan-TRK
11	Myofibroblastic sarcoma, intermediate grade[a]	13	*TFG-NTRK3*	Fascicular and/or herringbone with infiltrative growth	6	Patchy +	–	Patchy +	NP
12	Malignant spindle cell tumor[a]	10	*RBPMS-NTRK3*	Nuclear palisading and fascicular growth	6	Patchy +	+	Rare weak +	+, Cytoplasmic and nuclear
13	IF	0.2	*ETV6-NTRK3*	IMT-like	34	Patchy +	+	Rare +	+, Cytoplasmic and nuclear
14	IF	0.1	*ETV6-NTRK3*	Fasciitis-like, IMT-like, and focal fascicular growth	7	+	–	–	+, Cytoplasmic and nuclear
15	IF	0.5	*SPECC1L-NTRK3*	Infiltrative primitive cells, fibrosarcoma-like	25	+	+	–	+, Cytoplasmic and membrane
17	Spindle cell tumor, low grade[a]	0.5	*STRN3-NTRK3*	Mild to moderate cellularity with vascular proliferation and scattered plasma cells	2	–	+	–	Patchy +, Cytoplasmic and membrane

Abbreviations: HPF, high power field, IMT, inflammatory myofibroblastic tumor; NP, not performed.

[a]These tumors now meet criteria for the diagnosis of NTRK-rearranged spindle cell neoplasm according to the Fifth Edition of the Soft Tissue and Bone Tumors WHO Classification (2020).

be considered. Larotrectinib and entrectinib have shown antitumor effect for both primary brain tumors and solid tumors with brain metastases with systemic administration suggesting adequate penetration of the blood-brain barrier.[11] In a study of nine patients with primary CNS tumors treated with NTRK inhibitors, disease control was observed in all evaluable patients with stable disease in seven patients. Of note, there is overlap of three fusion genes between our CNS patient cohort and the study cohort (*KANK-NTRK2*, *SPECC1L-NTRK2*, and *ETV6-NTRK3*), suggesting that these patients may also derive benefit from larotrectinib in the setting of disease recurrence and/or progression.[14]

NTRK Fusions in Pediatric Soft-Tissue Tumors

Multiple soft-tissue tumors were identified with *NTRK3* rearrangements (Table 1) (Figs. 1 and 2A-C). There was a single case of cutaneous melanocytoma with epithelioid morphology containing *PRDX1-NTRK1* fusion (case 16). All other soft-tissue cases were spindle cell tumors with key morphologic and immunohistochemical findings described in Table 3. Some tumors exhibited typical age, morphology, and *ETV6-NTRK3* fusion compatible with IF, showing densely cellular fascicular growth of primitive ovoid cells and infiltration of surrounding tissue (cases 13 and 14). Other spindle tumors in the infantile age range (cases 15 and 17) showed similar morphology to IF, but contained variant *NTRK3* fusions (Table 3 and Fig. 2C). Notably, case 17 was initially not diagnosed as sarcoma because the lower cellularity and mitotic rate of that tumor did not suggest malignancy (Fig. 2C). Two spindle cell tumors in older children (cases 11 and 12) showed morphologic and immunohistochemical features of myofibroblastic sarcoma, one with marked nuclear palisading reminiscent of schwannoma (Table 3 and Fig. 2A). In all cases where performed, pan-TRK immunohistochemistry was positive (Table 3). Clinical classification and treatment based on histology only, particularly in soft-tissue tumors with spindle morphology, have proven to be challenging in some cases because of variable histologic features and immunohistochemical patterns.[30] As more tumors are being studied for fusions, the morphologic spectrum is expanding, such that the Fifth Edition of the Soft Tissue and Bone Tumors WHO Classification[31] now includes an emerging entity titled "NTRK-rearranged spindle cell neoplasm" to encompass spindle cell soft-tissue tumors with NTRK gene rearrangements (other than IF). It remains to be seen if soft-tissue histologic classification, molecular classification, or some combination of both will provide clinicians with the most accurate information for personalized treatment. Pathology diagnostic criteria will continue to shift as the field evolves to determine which features of a tumor are most prognostically significant for accurate classification. Despite the challenges and naming ambiguity, the detection of NTRK fusions is critical to providing targeted therapy in cases where resection would be morbid or impossible.[32] In our cohort, one IF (case 15) with a *SPECC1L-NTRK3* fusion was treated with larotrectinib and achieved complete tumor regression (Fig. 3). In a phase I or II study, two children with locally advanced IF displayed sufficient tumor shrinkage during larotrectinib treatment to allow for limb-sparing surgery and both remained progression-free without larotrectinib treatment after the follow-up of 4.8 and 6.0 months.[11] In another study of five children with locally advanced TRK fusion sarcomas, a median of six cycles of larotrectinib was given and all patients achieved partial response prior to surgical resection.[32] These studies and our experience showed that the use of TRK inhibitor can prevent pediatric patients from disfiguring surgery or amputation, reduce recurrence rate, and improve the quality of life.

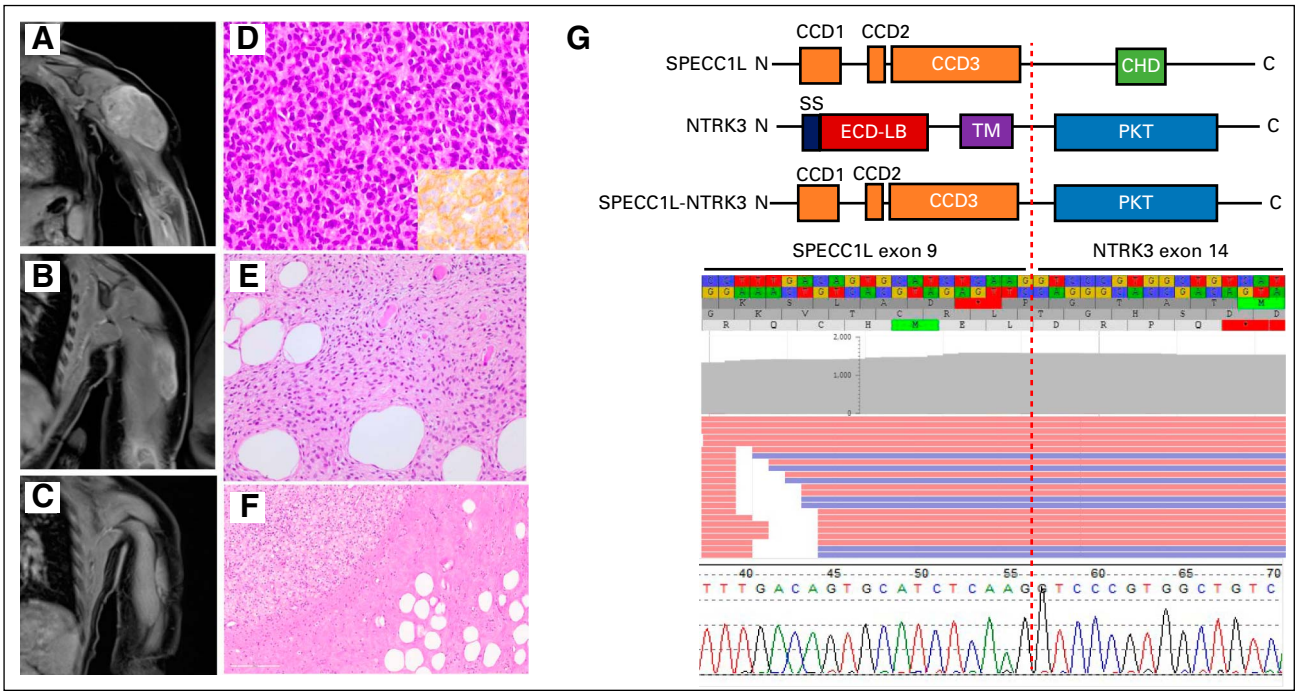

FIG 3. Case 15 with infantile fibrosarcoma with *SPECC1L-NTRK3* fusion. Magnetic resonance imaging at diagnosis (A), 1 month post-targeted therapy (B), and 5 months post-therapy (C) showing marked tumor shrinkage. At diagnosis, the tumor contained densely cellular areas with spindled to round cells and increased mitosis (D, H&E 400×) with cytoplasmic and membranous pan-TRK immunohistochemistry (inset). Other areas at diagnosis were less cellular with lower mitotic rate and demonstrate fat and skeletal muscle infiltration (E, H&E 200×). Resection following targeted therapy showed fibrosis and foamy histiocytes; no residual tumor was identified (F, H&E 200×). Schematic diagram demonstrates protein domains of *SPECC1L-NTRK3* fusion, detailed fusion sequences, and Sanger confirmation (G). CCD, coiled-coil domain; CHD, calponin homology domain; ECD-LB, extracellular ligand binding domain; H&E, hematoxylin and eosin staining; PKT, protein Tyr kinase; TM, transmembrane domain; SS, signal sequence.

NTRK Fusions Facilitate Precision Diagnosis, Prognosis, and Therapy

Follow-up information is available for all patients, and follow-up times ranged from 6 to 46 months. In almost all cases, the detection of an NTRK fusion confirmed the morphologic diagnosis, and in five cases, the final tumor diagnosis was largely based on the discovery of an NTRK fusion (excluding other differential diagnostic considerations). One exception was the case of secretory carcinoma, which was initially diagnosed as mucoepidermoid carcinoma, but later changed to secretory carcinoma following the detection of the *ETV6-NTRK*3 and additional immunohistochemical evaluation (case 10). Although the morphologic spectrum of NTRK fusion tumors is expanding, these fusions were not detected in certain tumor subtypes. Collectively, we analyzed 261 common embryonal solid tumors (79 neuroblastomas, 29 medulloblastomas, 28 Wilms tumors, 13 atypical teratoid/rhabdoid tumors, and 11 hepatoblastomas), bone tumors (27 osteosarcomas and 24 Ewing sarcomas), and skeletal muscle tumors (50 rhabdomyosarcomas), and none had NTRK fusions. Thus, although it is difficult to exclude the possibility that NTRK fusions might occur in individual tumors of these subtypes, which accounted for about one third of all solid tumors in this cohort, they are likely to be rare.

Case 15 was a 6-month-old male infant with a left upper extremity mass. Histology findings suggested an intermediate- to high-grade mesenchymal neoplasm negative for an *ETV6* rearrangement by fluorescence in situ hybridization (FISH). A novel fusion gene *SPECC1L-NTRK3* (Fig. 3) and complicated CNAs were detected (Appendix Table A1). Given that surgical resection would have resulted in significant morbidity to the upper extremity musculature, the patient received neoadjuvant treatment with larotrectinib. The tumor shrank substantially, and a much more limited surgical resection was then performed after six cycles of treatment, which showed no residual tumor (Figs. 3B-C). The patient continued on larotrectinib postsurgery for six additional cycles. Magnetic resonance imaging 6 months after the surgery demonstrated no residual or recurrent mass. Prospectively, the rest of the patients who survived with the standard therapy may also benefit from TRK inhibitor therapy if their tumors progress or recur.

We assessed the clinical outcome of all NTRK fusion–positive patients for up to 46 months (median 21 months). All non-CNS, non-PTC solid tumors showed excellent outcome after surgical resection with or without postsurgery radiation or chemotherapy at a median follow-up of 32.5 months. The median follow-up time for patients with PTC was 21.5 months. Of the 6 *ETV6-NTRK3*–positive PTCs, none

have progressed to date and three achieved remission with or without receiving RAI therapy. Of the four *NTRK1* PTCs, three of the four did not achieve remission with RAI. The median follow-up time for patients with CNS tumors was 21 months. The majority of NTRK-positive LGG demonstrated superb outcome with gross total resection without additional therapy. One patient with congenital glioblastoma and an *ETV6-NTRK3* fusion died 3 months after birth, and the tumor specimen was obtained via autopsy. The prognostic significance of NTRK fusions in hematological malignancies is not clear. The patient with T-lymphoblastic lymphoma and a *RBPMS-NTRK3* fusion achieved complete remission with standard chemotherapy and remains in remission at a 21-month follow-up. The patient with myeloid sarcoma and a *TPM3-NTRK1* fusion died during induction therapy. Evaluating other genomic alterations in NTRK fusion–positive tumors in this cohort, we found that SNVs are enriched in CNS tumors and hematological malignancies, whereas CNAs are mainly observed in non-CNS solid tumors (Fig. 1, Appendix Table A1). Although none of these co-occurring alterations are known drivers in these tumors, they may alter disease prognosis and/or play a role in the durability of and resistance to TRK inhibitors.

In summary, we studied the spectrum of NTRK-positive pediatric tumors in the largest cohort of unselected pediatric tumors to date and identified NTRK fusions in 2.22% of all tumors and 3.08% of solid tumors. Our review of 1,217 patients showed that NTRK fusions are more frequently seen in pediatric tumors than in adult tumors and involve a broader panel of fusion partners and a wider range of pediatric tumors than previously recognized. The identification of these NTRK fusions has facilitated precision cancer diagnosis and TRK inhibitor–targeted therapy. Our experience highlights the clinical utility of screening NTRK fusions for all pediatric tumors.

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APPENDIX

TABLE A1. Co-Occurring Molecular Findings of NTRK Fusion–Positive Tumors

Pt ID	Fusion Gene	3′ partner[a]	5′ partner[a]	SNVs/Indels[a]	CNAs
1	*KANK1-NTRK2*	*KANK1* (NM_015158.3) exon 3	*NTRK2* (NM_006180.4) exon 12	Not Identified	Anaplastic regions: loss of partial chr 9p including CDKN2A/B; gain of partial chr 9p including *JAK2*, *CD274*, and *PAX5*; and gain of partial 9q including *GNAQ*, *NTRK2*, *ABL1*, and *TSC1*.
2	*C2orf44-NTRK2*	*C2orf44* (NM_025203.2) exon 4	*NTRK2* (NM_006180.4) exon 16	Not identified	Not identified
3	*QKI-NTRK2*	*QKI* (NM_006775.2) exon 6	*NTRK2* (NM_006180.3) exon 16	*SETD2* (NM_014159.6), c.5287_5290del (p.Q1764Pfs*3) (VAF = 0.11)	Not identified
4	*KCTD16-NTRK2*	*KCTD16* (NM_020768.3) exon 3	*NTRK2* (NM_006180.4) exon 16	*CHEK2* (NM_007194.3), c.470T>C, (p.I157T) (VAF = 0.44)	Not identified
5	*TRIM24-NTRK2*	*TRIM24* (NM_003852.3) exon 12	*NTRK2* (NM_006180.4) exon 16	Not identified	Not identified
6	*SPECC1L-NTRK2*	*SPECC1L* (NM_015330.4) exon 6	*NTRK2* (NM_006180.4) exon 12	*ATM* (NM_000051.3), c.2921+1G>A, (p.?) (VAF = 0.51)	Loss of chr 22 and gain of partial chr 6p including *IRF4*, *TPMT*, *HIST1H3B*, and *HIST1H1C*.
7	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 4	*NTRK3* (NM_002530.3) exon 14	*CDKN1B* (NM_004064.4), c.25G>A (p.G9R) (VAF = 0.44)	Not identified
8	*RBPMS-NTRK3*	*RBPMS* (NM_006867.3) exon 5	*NTRK3* (NM_002530.3) exon 14	*NOTCH1* (NM_017617.3), c.4745_4746insAGACTTCCC (p.P1582_E1583insDFP) (VAF = 0.14) *BCL11B* (NM_138,576.3), c.1727_1739del (p.V576Gfs*25) (VAF = 0.12)	cnLOH of partial chr 9p.
9	*TPM3-NTRK1*	TPM3 (NM_152,263.3) exon 8	*NTRK1* (NM_002529.3) exon 12	*GATA2* (NM_001145661.1), c.1085G>A, (p.R362Q) (VAF = 0.51)	Not Identified
10	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 5	*NTRK3* (NM_002530.3) exon 15	*FANCC* (NM_000136.2), c.355_360delinsA (p.S119Nfs*8) (VAF = 0.47)	Gain of chr 3; loss of partial chr 4 including *FGFR3*; gain of chr 7; loss of partial chr 9p including *CDKN2A/B*; gain of partial chr 12p including *KDM5A* and *CCND2*; gain of partial chr 15q including *MAP2K1* and *NTRK3*; and loss of chr 22
11	*TFG-NTRK3*	*TFG* (NM_006070.5) exon 6	*NTRK3* (NM_002530.3) exon 14	Not identified	Loss of partial chr 3q including *GATA2*, *EPHB1*, and *FOXL2*; loss of partial chr 5p including *SDHA*, *TERT*, *IL7R*, and *RICTOR*; gain/loss of partial chr 7q; loss of partial chr 9p; loss of partial chr 10p/q; LOH of partial chr 11q; and loss of partial chr 15q including *IDH2* and *BLM*
12	*RBPMS-NTRK3*	*RBPMS* (NM_006867.3) exon5	*NTRK3* (NM_002530.3) exon 14	Not identified	Low-level mosaic triploidy of chr 1, 2, 3, 7, 10, 12, 13, and 18; four copies of chr 6 and 15; loss of partial chr 16p (2-3 copies); loss of chr 19 (2-3 copies); and cnLOH of partial Xp (two copies).
13	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 5	*NTRK3* (NM_002530.3) exon 15	Not identified	Gain of chr 17

(Continued on following page)

TABLE A1. Co-Occurring Molecular Findings of NTRK Fusion–Positive Tumors (Continued)

Pt ID	Fusion Gene	3′ partner[a]	5′ partner[a]	SNVs/Indels[a]	CNAs
14	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 5	*NTRK3* (NM_002530.3) exon 15	Not identified	Not identified
15	*SPECC1L-NTRK3*	*SPECC1L* (NM_015330.4) exon9	*NTRK3* (NM_002530.3) exon 14	Not identified	cnLOH of partial chr 3p including *SETD2*, *RHOA*, *BAP1*, and *PBRM1*; loss of partial chr 9p including *ABL1* and *TSC1*; loss of chr 11p and 14q; complex CNV for partial chr 15q including loss of exons 1-11 of *NTRK3* and gain of exons 12-18 of *NTRK3*; complex CNV of partial chr 17q including loss of *BRCA1* and gain of *SPOP*, *RNF43*, *PPM1D*, *BRIP1*, *CD79B*, *PRKAR1A*, and *RPTOR*; complex CNV of partial chr 22q including gain of *CRKL*, *MAPK1*, and *SMARCB1*; and loss of *EP300*.
16	*PRDX1-NTRK1*	*PRDX1* (NM_002574.3) exon 5	*NTRK1* (NM_002529.3) exon 12	*MUTYH* (NM_012222.2), c.1178G>A (p.G393D) (VAF = 0.46)	Loss of partial chr 1p, chr 9, and partial chr 15q.
17	*STRN3-NTRK3*	*STRN3* (NM_014574.3) exon 3	*NTRK3* (NM_002530.3) exon 14	Not identified	Not identified
18	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 5	*NTRK3* (NM_002530.3) exon 14	Not identified	Loss of partial chr 15q including *IDH2*, *BLM*, and *IGF1R*.
19	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 4	*NTRK3* (NM_002530.3) exon 14	*TET2* (NM_001127208.2), c.5152G>T (p.V1718L) (VAF = 0.48)	Not identified
20	*IRF2BP2-NTRK1*	*IRF2BP2* (NM_182,972.2) exon 2	*NTRK1* (NM_002529.3) exon 10	Not identified	Not identified
21	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 4	*NTRK3* NM_002530.3) exon 14	Not identified	Not identified
22	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 4	*NTRK3* (NM_002530.3) exon 14	Not identified	Not identified
23	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 5	*NTRK3* (NM_002530.3) exon 14	N/A (solid part canceled because of poor sample quality)	N/A (solid part canceled because of poor sample quality)
24	*SQSTM1-NTRK1*	*SQSTM1* (NM_003900.4) exon 4	*NTRK1* (NM_002529.3) exon 12	Not identified	Not identified
25	*TPR-NTRK1*	*TPR* (NM_003292.2) exon 21	*NTRK1* (NM_002529.3) exon 12	Not identified	Not identified
26	*TPR-NTRK1*	*TPR* (NM_003292.2) exon 9	*NTRK1* (NM_002529.3) exon 12	Not identified	Not identified
27	*ETV6-NTRK3*	*ETV6* (NM_001987.4) exon 4	*NTRK3* (NM_002530.3) exon 14	Not identified	Not identified

Abbreviations: Chr, chromosome; VAF, variant allele frequency.
[a]Based on GRCh37.

